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PATENT
Our Docket: P-IX 1653

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Kauffman and Ballivet	Group Art Unit: 1805
	Examiner: T. Wai
Serial No.: 08/464,142	
Filed: June 5, 1995	
For: PROCESS FOR OBTAINING DNA, RNA, PEPTIDES, POLYPEPTIDES, OR PROTEIN BY RECOMBINANT DNA TECHNIQUE	

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By _____
David A. Gay, Reg. No. 39,200

June 10, 1996
Date of Signature

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

AMENDMENT AND RESPONSE UNDER 37 C.F.R. §§ 1.111 and 1.115

This Amendment is submitted in response to the Office Action mailed December 8, 1995, in connection with the above-identified application. Applicants respectfully request entry of the following amendments and consideration of remarks below.

AMENDMENTS

Please amend the above-captioned application as follows:

In The Claims:

45. (Once amended) A process for the production of an expression vector which comprises [includes] at least one stochastic sequence of polynucleotide [polynucleotides], comprising the steps of:

providing [in a common milieu,] in an appropriate buffer at least three different sequences of oligonucleotides, said oligonucleotides each comprising at least 7 [nucleotides] nucleotide residues,

polymerizing said oligonucleotides [nucleotides to form] in a manner to form a stochastic sequence of polynucleotide [double stranded DNA fragment], and

ligating said stochastic sequence of polynucleotide [double stranded DNA fragments] into [an] a linearized expression vector.

2 ~~46~~. (Once amended) The process according to claim ~~45~~ wherein the oligonucleotides further comprise [are heptamers] a heptamer.

47. (Once amended) The process according to claim 45 [46 wherein the transforming DNA] said process further comprising the steps of:

transforming a competent clone with said ligated expression vector;

amplifying said transformed competent clone;

purifying [derived from a culture of clones] said expression vector from said amplified competent clone;

isolating [is isolated and purified,] said amplified stochastic sequence of polynucleotide from said expression vector;

cutting said stochastic sequence of polynucleotide [the purified DNA is cut] by means of at least one restriction enzyme which corresponds to a specific restriction enzyme site present in [the palindromic heptamers or octamers but absent from the expression vector,] said stochastic sequence of polynucleotide;

treating said cut [then the ensemble of linearized] stochastic [DNA fragments] sequence of polynucleotide [obtained are simultaneously treated] with a T4 ligase in manner to create a new ensemble of [DNA] stochastic sequence of polynucleotide containing a new stochastic sequence of polynucleotide [sequences,]; and

ligating said new ensemble of stochastic sequence of polynucleotide into an expression vector [this new ensemble of DNA is used to transform host cells].

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5 ~~45~~. (Once amended) The process according to claim ~~46~~ wherein the palindromic heptamers are selected from the group consisting of:

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5' XTCGCGA 3'[.]

5' XCTGCAG 3'[.] and

5' RGGTACC 3'[.]

where X = A, G, C, or T, and R = A or T.

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6 ~~50~~. (Once amended) The process according to claim ~~45~~ [46] wherein the oligonucleotides further comprise [are octamers] an octomer.

51. (Once amended) The process according to [Claim] claims ~~45~~ or 50 [wherein the transforming DNA], said process further comprising the steps of:

transforming a competent clone with said ligated expression vector;

amplifying said transformed competent clone;

purifying and isolating [derived from a culture of clones is isolated and purified,] said expression vector from said amplified competent clone;

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cutting said stochastic sequence of polynucleotide [the purified DNA is cut] by means of at least one restriction enzyme which corresponds to said [a] specific restriction enzyme site present in [the palindromic heptamers or octamers but absent from the expression vector,] said stochastic sequence of polynucleotide;

treating said cut [then the ensemble of linearized] stochastic [DNA fragments] sequence of polynucleotide [obtained are simultaneously treated] with a T4 ligase in manner to create a new ensemble of [DNA] stochastic sequence of polynucleotide [containing] comprising a new stochastic sequence of polynucleotide [sequences,]; and

ligating said new ensemble of stochastic sequence of polynucleotide into an expression vector [this new ensemble of DNA is used to transform host cells].

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9. ~~55.~~ (Once amended) The process according to claim ~~52~~ 8 wherein the palindromic octamers are selected from the group consisting of:

5' GGAATTCC 3';

5' GGTCGACC 3';

5' CAAGCTTG 3';

5' CCATATGG 3'; and

5' CATCGATG 3'.

55. (Once amended) A process for the production of an expression vector capable of producing a transcription product or a translation product [which includes] comprising at least one stochastic sequence of polynucleotide [polynucleotides], comprising the steps of:

linearizing an expression vector[,];

reacting said linearized expression vector with terminal transferase enzyme in the presence of desired ratios of deoxynucleotide-triphosphates of guanine, cytosine, thymidine, and adenine to form a stochastic polynucleotide sequence at each 3' extremity of said linearized vector [polymers,];

hybridizing said stochastic polynucleotide sequence at a 3' extremity of said linearized vector [polymers,]; and

synthesizing a second strand from said 3' ends of said hybridized vector [treating said polymers to form expression vectors consisting of stochastic double stranded DNA].

56. (Once amended) A process for the production of [an] a library of expression vectors [vector] capable of producing a transcription product or a translation product, said vectors comprising at least one stochastic sequence of polynucleotide, comprising the steps of:

producing [a library expression vectors which include] at least one stochastic sequence of polynucleotide [polynucleotides,];

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ligating said stochastic sequence of polynucleotide into an expression vector;

[introducing the] transforming a competent clone with said ligated expression vector [vectors,];

[cultivating the host cells containing these expression vectors so as to clone the expression vector and lead to the production of peptides, polypeptides, or proteins expressed by at least some of these expression vectors,] amplifying said transformed clone;

[carrying out] screening and/or selecting [selection methods on such] said transformed clone [clones] in order to isolate a clone expressing a stochastic polynucleotide leading to the synthesis of a transcription product or a translation product [producing the peptide, polypeptide, or protein having the predetermined property,];

and

isolating said selected or screened transformed clone;

isolating the [cloned] expression vector amplified in said selected or screened transformed clone so identified.

13 ~~62~~. (Once amended) A library of expression vectors capable of producing a transcription product or a translation product, said expression vectors comprising at least one stochastic sequence of polynucleotides. [comprising stochastic polynucleotide sequences which encode at least 10,000 peptides, polypeptides or proteins] produced in accordance with the process of claim 12 ~~55~~.

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Please add the following new claims:

--Claim 15 ~~67~~. (NEW) The process according to claims 11 ~~55~~ or 12 ~~56~~ wherein said translation product comprises a product having a desired property and is selected from the group consisting of a peptide, a polypeptide or a protein.--

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--Claim 16 ~~68~~. (NEW) The process according to claims 1 ~~55~~ or 12 ~~56~~ wherein said transcription product comprises a product having a desired property and is selected from the group consisting of a RNA or a DNA.--

--Claim 17 ~~69~~. (NEW) The library of expression vectors according to claim 13 ~~62~~ wherein said library comprises stochastic nucleotide sequences encoding for at least 10,000 peptides, polypeptides or proteins.--

REMARKS

A. Status Of The Claims

Claims 1 to 44, 57 to 61 and 64 to 66 are canceled. Claims 45 to 56, 62 and 63 are pending. New claims 67 to 69 have been added.

B. Summary Of The Pending Claims

Claims 45 to 54 and 55, as amended, claim a process for the production of an expression vector which includes at least one stochastic polynucleotide sequence. Claim 56 claims a process for the production of a library of expression vectors which includes at least one stochastic sequence of polynucleotide.

C. Support for the Amendments

The Specification sets forth an extensive description of the invention in the new and amended claims, and, for example, support may be found, *inter alia*: in the language of the specification and the Abstract; pages 2 to 3, lines 20 to 24 and 1 to 3, respectively; page 4, lines 9 to 24; page 10 to 11, lines 5 to 24 and 1 to 24, respectively; page 13, lines 18 to 24; pages

11 to 12, lines 13 to 24 and 1 to 14, respectively; page 16, lines 7 to 22; page 18, lines 8 to 20. Applicants respectfully submit that the subject matter encompassed by the new and amended claims are adequately described in the Specification as filed. Accordingly, the amendments do not raise an issue of new matter.

D. Formalities

Applicants thank the Examiner for acknowledging receipt of a certified copy of the priority document and that it has been filed in the parent application 06/942,630.

E. Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 45 to 56 and 63 stand rejected in the Office Action as being indefinite under 35 U.S.C. § 112, second paragraph. Applicants respectfully traverse this rejection.

The Office Action has alleged that the recitation of "in a common milieu" lacks specific meaning in the art. Applicants contend that this term is clear and descriptive to those skilled in the art. However, the claims have been amended above to further prosecution of the above-identified application. Therefore, any indefiniteness of claim 45 is resolved by the instant amendment.

Applicants thank the Examiner for noting that phrases in claims 47 and 51 lack antecedent basis. This issue has been resolved by the instant amendment.

F. Rejection of the Claims Under 35 U.S.C. § 103

Claims 45 to 56 and 62 to 63 stand rejected under 35 U.S.C. § 103, as allegedly obvious over Sirotkin, K.M., U.S. Patent No. 4,959,312 (hereinafter "Sirotkin"). Applicants respectfully traverse this rejection.

In this rejection, Sirotkin is alleged to contain deficiencies that are cured by general knowledge known to one skilled in the art. However, Applicants contend the deficiencies in Sirotkin are not cured by such general art. Accordingly, a *prima facie* case of obviousness has not been established and the rejection should be properly withdrawn.

The Office Action relies on Sirotkin for allegedly describing: a method of producing random sequences using terminal transferase and a method that produces mutants containing random substitution mutations at random sites in a DNA sequence; that the new sequence may be amplified by cloning the sequence into a cloning vector; the use of T4 ligase to extend random primers and to seal nicks. The Office Action further alleges that since

Sirotkin teaches a method of producing expression vectors containing randomly generated nucleotide sequences, it renders the claimed invention ("the method such as individual expression vectors and a library of expression vectors") obvious.

A *prima facie* case of obviousness is established only when the teaching from the prior art itself would appear to have suggested the claimed subject matter to a person of ordinary skill in the art. The art must suggest how to apply the teachings to the specifically claimed invention. Sirotkin makes no mention of the claimed methods of producing populations of stochastic molecules, including expression vectors and libraries containing stochastic polynucleotide sequences. Such claimed populations are diverse in sequence and complexity and are produced by, for example, the random copolymerization or chemical coupling of nucleotide monomers. None of the cited art or general knowledge contains any specific suggestion to create stochastic sequences independent of "target sequences."

In contrast, Sirotkin is directed to the mutagenesis of a "target" DNA. The methods described by Sirotkin are for mutagenizing known DNA sequences and result in random substitution mutations or insertions within a known sequence,

described as a "target sequence."[1] The methods of Sirotkin generate mutants containing a single, randomly-generated region in the target sequence with random substitution or addition mutations. However, such methods do not result in or suggest the production of random, stochastic sequences as claimed in this invention. General knowledge at the time of the invention did not teach or suggest the production of such random, stochastic sequences. Therefore, Sirotkin cannot render the claimed invention obvious and general knowledge at the time of the invention cannot cure Sirotkin's deficiencies. Accordingly, Applicants respectfully assert that a combination of Sirotkin and general knowledge at the time of the invention would not suggest how to apply these teachings to invent a diverse population of random, stochastic polynucleotide sequences contained within expression vectors and libraries of such expression vectors.

The Patent Office bears the burden of establishing a *prima facie* case of obviousness based upon the prior art.[2] The Patent Office can satisfy this burden only by showing some

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[1] The abstract of Sirotkin reads "A method is disclosed for in vitro mutagenesis of a target DNA sequence which generates mutants containing a single randomly-generated region in the target sequence with random substitution mutations ..." (emphasis added)

Claim 1 of Sirotkin reads: "An in vitro method of mutagenesis of a target DNA sequence comprising: producing a supply of a template for the target sequence; ..." (emphasis added)

[2] *In re Piasecki*, 745 F.2d 1468, 223 U.S.P.Q. 785, 787-88 (Fed. Cir. 1984).

objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teaching of the references.[3] "Under section 103, teachings of references can be combined only if there is some suggestion or incentive to do so." [4] In other words, there must be a suggestion in the references, or the general art, cited by the Office Action, to do what the Applicants have done.[5]

In *Obukowicz*, the Board reversed an obviousness rejection against an invention concerning a method for combating plant insect pests by inserting heterologous DNA encoding insect toxin genes into a *plant-growing* bacteria. The cited art disclosed incorporation of the same insect toxin genes into *enteric* bacteria such as *E. coli*. The cited art also suggested the transfer of genes toxic to insects into "other bacteria" which have a better rate of survival in nature and insertion of these genes into the plant itself. However, the Board dismissed this reference stating that it "contains little information regarding how to use the transformed bacteria and clearly does

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[3] *In re Fine*, 837 F.2d 1071, 1074, 4 U.S.P.Q. 2d 1596, 1598 (Fed. Cir. 1988).

[4] *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 723 F.2d 1572, 1577, 221 U.S.P.Q. 929, 933 (Fed Cir. 1984).

[5] *Ex parte Obukowicz*, 27 U.S.P.Q.2d 1063, 1065 (BPAI 1993). (emphasis in original)

not specifically suggest appellant's use [inserting heterologous DNA encoding insect toxin genes into a *plant-growing* bacteria]."[6] (emphasis added)

Similarly, as noted above, neither Sirotkin nor any general knowledge specifically suggest Applicant's invention, *i.e.* production of expression vectors and libraries containing stochastic sequences. The Board in *Obukowicz* also stated:

> At best, the [cited art] is but an invitation to scientists to explore a new technology that seems a promising field of experimentation. The [cited art] is of the type that gives only general guidance and is not at all specific as to the particular form of the claimed invention and how to achieve it. Such a suggestion may make an approach "obvious to try" but it does not make the invention obvious.[7]

The same can be said for the art and general knowledge cited in the instant Office Action. As in *Obukowicz*, none of the

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[6] *Obukowicz* 27 U.S.P.Q.2d at 1065.

[7] ... *In re O'Farrell*, 853 F.2d 894, 7 U.S.P.Q.2d 1673, 1681 (Fed Cir. 1988). . . one can theoretically explain the technological rationale for the claimed invention using selected teachings from the references. This approach, however, has been criticized by our reviewing court as hindsight reconstruction. See *In re Fine*, supra, 837 F.2d at 1075, 5 U.S.P.Q.2d at 1600. See also *In re Sernaker*, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983) (emphasis added) *Obukowicz* 27 U.S.P.Q.2d at 1065.

cited art or general knowledge contains any specific suggestion to create expression vectors and libraries containing stochastic sequences. None of the cited art or general knowledge contains any specific suggestion to create stochastic sequences independent of "target sequences." As in *Obukowicz*, the combination of cited art and general knowledge is, *at best*, an invitation to explore a field of experimentation. The combination of cited art and general knowledge is not specific as to the particular form of the claimed invention, namely expression vectors and libraries of expression vectors specifically for expressing stochastic sequences. General guidance does not make an invention obvious.[8] Accordingly, a case of *prima facie* obviousness using Sirotkin and general knowledge could not be established.

In view of the above, this rejection of the claims under 35 U.S.C. § 103 should be withdrawn.

Claims 45 to 56, 62 and 63 also stand rejected under 35 U.S.C. § 103, as allegedly obvious over Gilbert, W., et al., U.S. Patent No. 4,338,397 (hereinafter "Gilbert"), in view of Wu,

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[8] Use of the prior art to theoretically explain the technological rationale for the claimed invention or to "point in the direction of" the claimed subject matter is impermissible hindsight reconstruction. *In re Fine*, 837 F.2d at 1075, 5 U.S.P.Q.2d at 1599-1600.

R.J., et al., U.S. Patent No. 4,321,365 (hereinafter "Wu").

Applicants respectfully traverse this rejection.

In this rejection Gilbert is alleged to contain deficiencies that are cured by Wu or general knowledge known to one skilled in the art. Applicants will show that the deficiencies in Gilbert are not cured by Wu or such general art. Accordingly, a *prima facie* case of obviousness has not been established and the rejection should be properly withdrawn.

The Office Action relies on Gilbert for allegedly teaching a method of transforming a cloning vehicle that contains a foreign DNA fragment into a host cell and then culturing the transformed host so the selected protein or polypeptide is secreted. Gilbert does not teach or suggest, in fact makes no mention of, the production of stochastic sequences, expression vectors or libraries of expression vectors specifically for the purpose of expressing such stochastic sequences. Furthermore, Gilbert does not teach or suggest the generation of any stochastic sequences or use of any stochastic process.[9]

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[9] The Abstract of Gilbert reads "A method is provided for synthesizing within a bacterial host, and secreting through the membrane of the host, a selected mature protein or polypeptide..."

The Office Action relies on Wu for curing the deficiencies in Gilbert. Wu is cited for allegedly teaching the use of chemically synthesized oligonucleotides that have nucleotide sequences which are the recognition sites for restriction endonucleases, and that these adaptor molecules are joined to natural or synthetic DNA molecules, then joined to a cloning vehicle. However, Wu does not teach or suggest, in fact makes no mention of, the production of expression vectors or libraries of expression vectors specifically for expressing such stochastic sequences. Furthermore, Wu does not teach or suggest any stochastic sequence or stochastic process. Wu introduces adaptor molecules, whose sequences are known, into cloning vehicles for the purpose of adding restriction sites or identifying markers.[10]

The Office Action alleges that it would have been obvious to clone any sequence, whether or not it was stochastically generated, with the synthetic adaptor molecules taught by Wu, and cloned the resulting segment of DNA into a cloning vehicle and transformed a host cell so that the selected protein or polypeptide was secreted. However, the generation of

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[10] Wu's invention is summarized in the abstract: "Synthetic oligonucleotides have been designed and prepared which are useful in the molecular cloning of a variety of DNA molecules ..."; and, on page 2, lines 36 to 49, Detailed Description of the Invention, Adaptor Molecules, "Adaptor molecules for the insertion of genetic informational material, e.g. DNA, into cloning vehicles have been prepared ..".

stochastic sequences is one inventive concept in the claimed invention. Thus, any reference or combination of references which only teach the cloning of known sequences or the modification of known ("target") sequences cannot render the instant invention obvious. None of the cited art or general knowledge contain any specific suggestion to create expression vectors and libraries of expression vectors for the purpose of expressing the random, stochastic sequences of the claimed invention. None of the cited art or general knowledge contains any specific suggestion to create stochastic sequences independent of a "target sequences." Accordingly, because Wu and general knowledge at the time of the invention cannot cure the deficiencies of Gilbert, a case of *prima facie* obviousness using Gilbert, Wu and general knowledge cannot not be established.

Applicants respectfully submit that in view of the above presented arguments, the rejection of the claims under 35 U.S.C. § 103 should be withdrawn.

CONCLUSION

In light of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the objection to the specification and rejections to the claims under 35 U.S.C. § 112, second paragraphs and 35 U.S.C. § 103. Applicants contend that the present application is now in condition for immediate allowance. The Examiner is invited to contact Cathryn Campbell or the undersigned agent at (619) 535-9001 if there are any issues yet to be resolved.

Respectfully submitted,

Date: June 10, 1996

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